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Adenosine A₁ Receptors in the Central Nervous System: Their Functions in Health and Disease, and Possible Elucidation by PET Imaging

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Abstract: Adenosine is a neuromodulator with several functions in the central nervous system (CNS), such as inhibition of neuronal activity in many signaling pathways. Most of the sedating, anxiolytic, seizure-inhibiting and protective actions of adenosine are mediated by adenosine A₁ receptors (A₁R) on the surface of neurons and glia. Positron Emission Tomography (PET) is a powerful *in vivo* imaging tool which can be applied to investigate the physiologic and pathologic roles of A₁R in the human brain, and to elucidate the mechanism of action of therapeutic drugs targeting adenosine receptors, nucleoside transporters and adenosine-degrading enzymes. In this review article, we discuss (i) functions of adenosine and its receptors in cerebral metabolism; (ii) radioligands for A₁R imaging: xanthine antagonists, non-xanthine antagonists, and agonists; (iii) roles of A₁R in health and disease, viz. sleep-wake regulation, modulation of memory retention and retrieval, mediating the effects of alcohol consumption, protecting neurons during ischemia and reperfusion, suppression of seizures, modulating neuroinflammation and limiting brain damage in neurodegenerative disorders. The application of PET imaging could lead to novel insights in these areas. Finally (iv), we discuss the application of PET in pharmacodynamic studies and we examine therapeutic applications of adenosine kinase inhibitors, e.g. in the treatment of pain, inflammation, and epilepsy.

Keywords: Adenosine, Alcohol Abuse, Alzheimer Disease, Brain, Drug Development, Epilepsy, Inflammation, Multiple Sclerosis, Pain, Parkinson's Disease, Positron Emission Tomography, Receptor Imaging, Sleep, Stroke.

1. INTRODUCTION

1.1. Sources, Regulation and Fate of Extracellular Adenosine

The purine nucleoside adenosine is not a classical signaling substance, as adenosine is neither stored nor released from vesicles. The nucleoside plays a neuromodulatory role by affecting the excitability of neurons that release neurotransmitters such as glutamate, γ -amino butyric acid (GABA), acetylcholine and dopamine [1-12]. Adenosine is involved in homeostatic reduction of cellular excitability during stress and trauma *via* its interaction with receptors on the cell surface. Moreover, adenosine is involved in the regulation of synaptic plasticity. Balanced activation of inhibitory and facilitatory adenosine receptors modulates long term potentiation (e.g., in the hippocampus) [13-15].

Metabolic pathways involved in the formation and removal of adenosine are presented in Fig. (1). Extracellular adenosine may originate from intracellular adenosine - which can pass the plasma membrane *via* an equilibrative nucleoside transporter (ENT), or by hydrolysis of locally released adenine nucleotides such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and to a lesser extent cyclic adenosine monophosphate (cAMP). Production of extracellular adenosine is the result of the breakdown of such nucleotides by ecto-nucleotidases [16-18]. The rate-limiting step is the conversion of AMP to adenosine which is catalyzed by ecto-5'-nucleotidase (CD73). The biochemical and pharmacological properties of this enzyme have been extensively characterized [16,19]. 5'-Nucleotidase is inhibited by ATP and ADP [18]. The quantitative importance of this mechanism has been confirmed by a kinetic characterization of the breakdown of extracellular ATP at the cholinergic striatal synapse [20].

Extracellular adenosine is subject to metabolic degradation by adenosine deaminase (ADA) which results in the formation of inosine [21]. Another route of adenosine inactivation is the reversible reaction catalyzed by S-adenosyl homocysteine (SAH) hydrolase [22]. Formation of SAH from adenosine and

homocysteine is restricted since homocysteine levels are rate-limiting. In the brain, the SAH pathway is a quantitatively negligible pathway of adenosine catabolism [23]. The major route of adenosine removal under normal conditions is phosphorylation to AMP by adenosine kinase (AK).

1.2. Classification of Adenosine Receptors

The adenosine receptor (AR) family consists of the A₁, A_{2A}, A_{2B} and A₃ subtypes (A₁R, A_{2A}R, A_{2B}R and A₃R, respectively). A₁R and A₃R inhibit whereas A_{2A}R and A_{2B}R stimulate production of the second messenger, cAMP. A₁R and A_{2A}R are activated by nanomolar concentrations of adenosine whereas A_{2B}R and A₃R become activated only when adenosine levels rise into the micromolar range during inflammation, hypoxia or ischemia [24-26]. The four AR subtypes from rat, mouse, human and other species have been pharmacologically recognized, cloned, purified and expressed [27-29]. In the following pages, we will focus on the A₁R and the potential contribution of positron emission tomography (PET) imaging to the study of its involvement in physiological processes.

1.3. Regional Distribution and Signaling of A₁R

The A₁R is the most strongly preserved AR subtype among different animal species [28]. A₁Rs exhibit the highest affinities for adenosine and synthetic agonists of all AR subtypes [30]. They are distributed throughout the body and are also highly expressed in the brain, especially in hippocampus, frontal cortex, thalamic nuclei, basal ganglia and dorsal horn of the spinal cord. Peripheral organs with high A₁R expression include eye, adrenal gland, heart and aorta.

The regional distributions of A₁R in human and rodent brains are similar but not completely identical. In human cerebellum, A₁R densities are low in contrast to rat cerebellum, where moderate A₁R expression is noted [31-36]. Different neurons express different levels of A₁R. In rat brain, the highest A₁R immunoreactivity is observed in pyramidal neurons of layer 5 of the cerebral cortex, and in pyramidal cells in fields CA2 and CA3 of the hippocampus. In layer 5, immunoreactivity is detected in cell bodies, dendrites and initial segments of axons [37].

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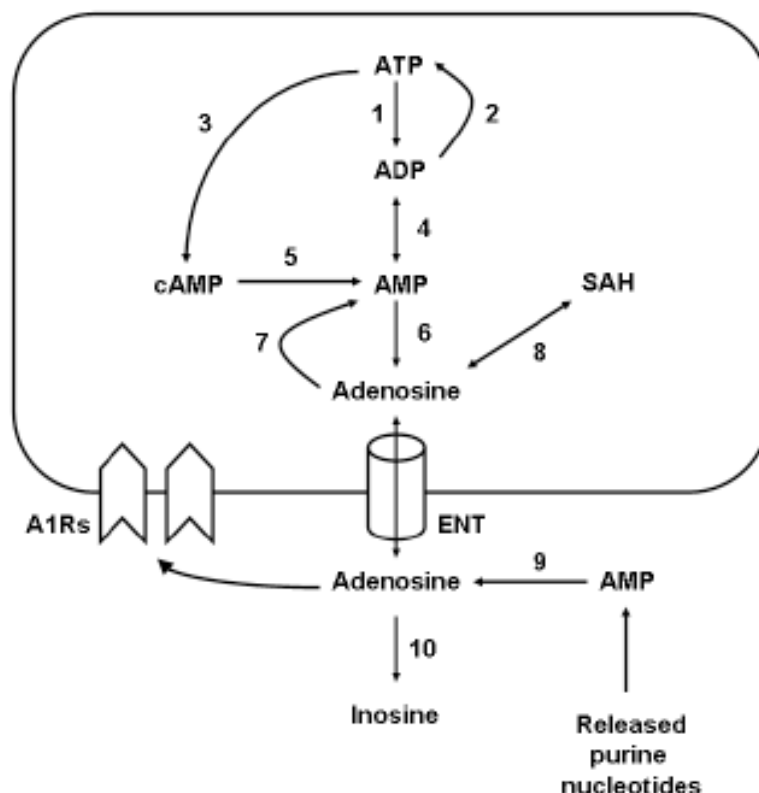


Fig. (1). Metabolic scheme showing the pathways of adenosine formation and removal. Enzymes involved are: 1. ATPases, 2. oxidative phosphorylation (and creatine kinase), 3. adenylyl cyclase, 4. adenylate kinase, 5. phosphodiesterase, 6. 5'-nucleotidase, 7. adenosine kinase, 8. SAH hydrolase, 9. ecto-5'-nucleotidase (CD73), 10. adenosine deaminase.

Activation of A_1R s inhibits adenylyl cyclase, closes voltage-dependent Ca^{2+} channels, and activates K^+ channels. Signalling of this AR subtype occurs through $G_{i/o}$ pathways in various cells (including neurons). As a result, the neuronal activity is suppressed [38,39]. In presynaptic regions, adenosine inhibits neurotransmitter and neuropeptide release, including the release of glutamate, substance P and calcitonin gene related peptide [40]. In postsynaptic areas, adenosine abolishes sensory transmission and causes membrane hyperpolarisation [41,42].

2. RADIOLIGANDS FOR A_1R IMAGING

PET is a medical imaging technique providing information on tissue biochemistry rather than anatomy. PET imaging has several unique properties: high sensitivity, low radiation dose, possibility to correct data for attenuation and scatter (thus quantitative), radioactive labeling of natural substances or drugs with high specific radioactivity so that these can be used as tracers to monitor the pharmacokinetics of the non-radioactive compounds. PET may therefore be applied to measure regional AR densities in the living human brain and the dose-dependent occupancy of cerebral AR by therapeutic drugs. A comprehensive overview of PET tracers for the different AR subtypes has been presented in some recent reviews [43,44].

Several ligands for PET imaging of A_1R have been prepared (see Table 1 and Fig. 2). These include xanthine A_1R antagonists, non-xanthine A_1R antagonists and A_1R agonists. All compounds bind with nanomolar affinity to A_1R . The best-characterized tracers are [^{11}C]MPDX and [^{18}F]CPFPX. Both can be applied for PET studies of cerebral A_1R in humans. The long physical half life of ^{18}F (109.8 min) as compared to ^{11}C (20.4 min) allows longer scanning times with [^{18}F]CPFPX than with [^{11}C]MPDX. Because of this

difference in half life, [^{18}F]CPFPX can be distributed to remote imaging sites without cyclotron facilities, in contrast to [^{11}C]MPDX. However, the radiation dose that subjects will receive after injection of [^{18}F]CPFPX (300 MBq) is greater than that of [^{11}C]MPDX (300 MBq): 5.3 mSv vs. 1.05 mSv, respectively ([45] and Ishiwata, unpublished data). Thus, multiple-injection protocols are possible with [^{11}C]MPDX but not with [^{18}F]CPFPX. Since each tracer has specific advantages and disadvantages, tracer selection will be based on historic interest and the facilities at one's disposal rather than on clear superiority of a certain compound.

Efforts to develop A_1R ligands for PET with improved properties might focus on antagonists with reduced lipophilicity and improved water solubility, e.g. compounds with non-xanthine structures. However, the first reported example of such tracers, [^{11}C]FR194921, did not produce better results in experimental animals than [^{11}C]MPDX or [^{18}F]CPFPX [46].

3. PHYSIOLOGICAL ROLE OF A_1R AND CHANGES OF A_1R EXPRESSION IN DISORDERS OF THE CNS

3.1. Sleep-Wake Regulation

Adenosine plays an important role in the induction of sleep after prolonged wakefulness [47]. Prolonged wakefulness with its associated prolonged neuronal activity increases extracellular adenosine levels in the forebrain of conscious cats, whereas these levels diminish during sleep [48]. A [^{18}F]CPFPX-PET study in humans has indicated that in addition to changes of extracellular adenosine, A_1R are upregulated in cortical and subcortical regions of the brain after prolonged wakefulness (max. 15.3%), which suggests that changes of A_1R expression are contributing to homeostatic sleep regulation [49]. Short periods of total sleep deprivation (3 or 6 h) result in increases of A_1R mRNA but no

Table 1. Development of Radioligands for PET Imaging of A₁R

Ligand	A ₁ R affinity	Studies performed	Findings	References
<i>Xanthines</i>				
[¹¹ C]KF15372 (8-Dicyclopropylmethyl-3-propylxanthine)	3.0 nM (K _i)	Biodistribution study in mice Ex vivo autoradiography (mice/rats) PET study in anesthetized monkeys	About 57% specific binding (to A ₁ R not A ₂ R) Tracer distribution in brain reflects regional A ₁ R density Decreased binding in superior colliculus after unilateral eye removal Tracer distribution in brain reflects regional A ₁ R density About 50% reduction in uptake after treatment with “cold” KF15372	[254-256]
[¹¹ C]EPDX (2-Ethyl-8-dicyclopropylmethyl-3-propylxanthine)	1.7 nM (K _i)	Biodistribution study in mice	About 50% specific binding (to A ₁ R not A ₂ R)	[257]
[¹¹ C]MPDX (8-Dicyclopropylmethyl-1-methyl-3-propylxanthine)	4.2 nM (K _i)	Biodistribution study in mice Metabolite analysis in mice Ex vivo autoradiography (rats) Radiochemical synthesis improved. PET study in anesthetized cats PET study in anesthetized monkey Human PET study (healthy volunteers)	Initial brain uptake higher than EPDX and KF15372 but faster washout. Dosimetry data indicate acceptable radiation dose in human studies. Metabolites appear in plasma but brain activity is mainly parent at 30 min. Decreased binding in superior colliculus after unilateral eye removal. About 55% specific binding (to A ₁ R not A ₂ R). In animal model of dystonia, tracer binding in hippocampus is decreased. --- Distribution volume of tracer in brain reflects regional A ₁ R density. Bound tracer can be displaced by an excess of cold A ₁ R antagonist. In a cat model of stroke, losses of A ₁ R can be detected in ischemic areas. The magnitude of these losses indicates severity of the insult and predicts subsequent complications (including mortality). Good brain uptake, distribution reflects regional A ₁ R density. Tracer distribution in brain reflects regional A ₁ R density. Pattern differs from that of a flow tracer or a glucose analog. Distribution volume (Logan plot) or binding potential (compartment model analysis) can be used for quantification purposes.	[257] [258] [257,259,260] [261] [262] [263] [155,156] [258] [264,265] [266]
[¹⁸ F]CPFPX (8-Cyclopentyl-3-[3-fluoropropyl]-1-propyl-xanthine)	0.6-1.4 nM (K _d mouse, pig, human) 4.4 nM (K _d rat)	Biodistribution study in mice Metabolite analysis in mice Ex vivo autoradiography (rats) Animal PET study in rats Human PET study (healthy volunteers) Metabolite study (liver microsomes)	Distribution in brain reflects regional A ₁ R density. Metabolites appear in plasma but brain activity is mainly parent at 60 min. About 70% of brain uptake is specific (to A ₁ R) and reversible. Tracer distribution in brain reflects regional A ₁ R density (>90% specific). Brain well-visualized, bound tracer can be displaced by A ₁ R antagonist. Tracer distribution in brain reflects regional A ₁ R density. Tracer kinetics in human brain are appropriate for quantitative imaging. Distribution volume (Logan plot) or binding potential (compartment model analysis) can be used for quantification purposes. Simplified study protocols are possible (venous rather than arterial blood sampling, bolus-infusion or single bolus administration of the tracer). Short scanning protocols (60 min) are possible in humans. Bound tracer can be displaced by cold CPFPX in all brain regions. Non-invasive procedure (reference tissue model) is suitable for quantification of A ₁ R in human brain. Tracer is metabolized by cytochrome CYP1A2. Its metabolism can be inhibited by therapeutic drugs like fluvoxamine.	[267] [268] [269] [270] [271,272] [273] [224] [274] [275,276]
[¹³¹ I]CPIPX ¹ (8-Cyclopentyl-3-[(<i>E</i>)-3-iodoprop-2-en-1-yl]-1-propylxanthine)	0.8-7.9 nM (K _d rat, pig cortex)	Ex vivo autoradiography (rats)	Tracer binding is largely nonspecific. Thus, this ligand is not suitable for imaging purposes. The iodine radiolabeling results also in loss of selectivity for the A ₁ R.	[277]
<i>Non-xanthines</i>				
[¹¹ C]FR194921 (2-(1-methyl-4-piperidiny)-6-(2-phenylpyrazolo[1,5-a]-pyridin-3-yl)-3(2H)-pyridazinone)	2.9 nM (K _i)	Ex vivo autoradiography (rats) PET study in conscious monkeys	Tracer distribution in brain reflects regional A ₁ R density. About 50% specific binding (to A ₁ R not A ₂ R). Brain well-visualized, tracer accumulates in cortex, striatum, thalamus.	[46] [46]
<i>Agonists</i>				
5-O-(methyl[⁷⁵ Se]seleno)-N ₆ -cyclopentyladenosine	0.9 nM (K _i) (pig cortex)	Radiochemical synthesis described	No <i>in vivo</i> data reported	[278]
5'-N-(2-[¹⁸ F]fluoroethyl)-carboxamidoadenosine	Nanomolar range	Radiochemical synthesis described	No <i>in vivo</i> data reported May bind not only to A ₁ R but also to other AR subtypes	[279]

¹This compound was prepared for Single Photon Emission Computed Tomography (SPECT) rather than PET imaging.

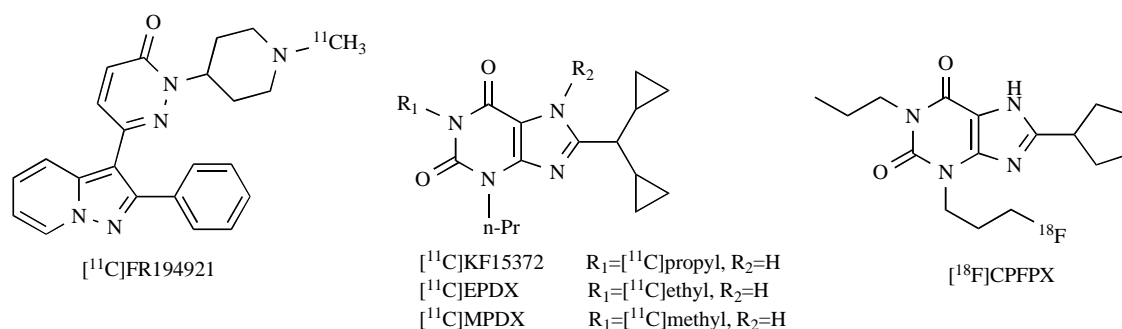


Fig. (2). Antagonist radioligands for PET imaging of A₁R.

detectable increases of A₁R expression in rat brain [50]. However, longer periods of total sleep deprivation in rodents (12 or 24 h) are accompanied by a significant upregulation of A₁R (up to 14%) in various brain areas [51,52]. [³H]Phenylisopropyladenosine (PIA, Fig. 3) saturation binding assays have indicated that A₁R densities in cortical and subcortical regions of rat brain are increased not only after total sleep deprivation, but also after 48 and 96 h of partial, rapid eye movement (REM) sleep deprivation, whereas affinity (K_d) values for agonist binding are not significantly altered [53]. After restriction of sleep and also after performance of a difficult learning task, cerebral synchronized slow wave activity (SWA) is enhanced during the next sleeping period, and this activity is related to the subsequent improvement of cognitive performance [54,55]. Activation of A₁R is necessary for the increase of SWA. If this increase is impaired (e.g. because of knockout of the A₁R gene) animals fail to maintain normal cognitive function. Thus, adenosine appears to be an important homeostatic sleep factor, and A₁Rs are involved in the regulation of sleep.

After administration of a selective A₁R agonist, N⁶-Cyclopentyladenosine (CPA, Fig. 3), either by the systemic or the cerebroventricular route, SWA increased in rats during non-REM sleep [56]. Such stimulation of A₁R mimics the effect of total sleep deprivation in rodents. A similar effect on sleep is observed when extracellular adenosine levels in the mammalian brain are raised by administering inhibitors of nucleoside transport [57]. When levels of extracellular adenosine in the basal forebrain are raised by microdialysis, vigilance of rats is significantly impaired [58]. This effect on vigilance is completely blocked after co-administration of a selective A₁R antagonist, 8-cyclopentyltheophylline (CPT, Fig. 3), with adenosine [58]. On the other hand, infusion of CPT into the basal forebrain diminishes the sleeping ability (both slow-wave and REM sleep) of cats [57]. Infusion of A₁R antisense in the cholinergic basal forebrain of rats inhibits the translation of A₁R mRNA, resulting in decreased non-REM sleep and increased wakefulness [59]. Such pharmacological experiments have provided additional support for the important role of A₁R in sleep regulation. Agonists for this receptor usually promote [60-62] while antagonists diminish [60,63] sleep.

In rat brain, prolonged wakefulness with accompanying increases of extracellular adenosine and stimulation of A₁R increases the activity of the transcription factor NF-κB [64,65]. This transcription factor regulates expression of several modulatory substances, such as interleukin-1-β and tumor necrosis factor α, which shows a diurnal rhythm. Some effects of adenosine on sleep homeostasis, particularly the phenomenon of "sleep debt" may be triggered via this mechanism.

Orexinergic neurons in the lateral hypothalamus also express A₁R [66]. Adenosine and A₁R agonists inhibit activity of the hypocretin/orexin neurons in this area via A₁R which promotes sleep [67,68]. On the other hand, blockade of A₁R in such neurons causes a pronounced increase in wakefulness [68,69]. Adenosine reduces the frequency of action potentials in hypocretin/orexin

neurons by depressing excitatory synaptic transmission to these cells. An additional mechanism involved in the sleep-promoting effects of adenosine may be suppression of the activity of histaminergic neurons in the tuberomammillary nucleus via A₁R [70]. Thus, in addition to its effects in basal forebrain, adenosine promotes sleep by reducing the activity of orexinergic and histaminergic neurons in the hypothalamus [67].

An additional brain region involved in sleep regulation is the pontine reticular formation. Microinjection of the A₁R agonist N⁶-cyclopentyladenosine (CPA) or inhibition of the formation of cAMP in this area increases REM sleep [71]. Another study has shown that administration of an A₁R agonist (N⁶-p-sulfophenyladenosine) decreases the (arousal-promoting) release of the neurotransmitter acetylcholine and increases the recovery time from halothane anesthesia [7].

Neurons in the previously mentioned brain areas are wake-active. Thus, suppression of their activity via stimulation of A₁R promotes sleep. In contrast to the regions mentioned above, the lateral preoptic area of rat hypothalamus is an area with an abundance of sleep-active neurons. A₁R agonists and the adenosine transport inhibitor 4-nitrobenzyl-thioinosine have opposite effects in this area as in other regions of the brain, i.e. they increase wakefulness rather than sleep [72]. Thus, the adenosine-mediated effects on the sleep-wake cycle are both neuron- and region-dependent.

PET may be applied to study the involvement of A₁R in the regulation of human sleep. Increased expression of A₁R in the human cortex after prolonged wakefulness has already been demonstrated [49]. Future PET studies could examine the effect of partial (REM) sleep deprivation in humans, and cerebral A₁R expression in sleeping disorders like narcolepsy.

3.2. Learning and Memory

Acute stimulation of A₁R by agonists at micromolar doses has been reported to severely impair both the acquisition and retention of memory in various animal and *in vitro* models, such as long-term potentiation in rat hippocampal slices [73], passive avoidance retention in mice [74,75], working memory in a three-panel test [76], and acquisition of conditional fear conditioning [77] or short-term social memory in rats [78]. These cognitive deficits are attenuated, or abolished, when brain slices or experimental animals are pretreated with selective A₁R antagonists such as DPCPX. Thus, activation of A₁R in many regions of the CNS (hippocampus, ventral striatum, posterior cingulate cortex) may negatively modulate information processing in the brain and may impair memory retention [73-76]. Depending on the test setup (dose, region of administration, time of the day), acute administration of a selective A₁R antagonist has either no effect or it can facilitate memory performance of rodents [75,78,79].

In contrast to acute administration, chronic administration of an A₁R agonist (daily i.p. injections of CPA during a 9-day period)

results in facilitation of spatial learning and memory of mice tested in a Morris water maze. These improvements are probably related to downregulation of A₁R [80].

However, studies of the behavioral phenotype of mice lacking A₁R have indicated that A₁R are not essential for rodent learning. Such mice show normal motor coordination but reduced muscle strength. Overall spontaneous motor activity is similar to that of wild-type controls, but activity peaks during the light/dark cycle are flattened, which is consistent with A₁R regulating the sleep-wake rhythm. A₁R^{-/-} mice show enhanced aggression and decreased exploratory behavior indicating increased anxiety. However, their working memory in five different water maze tasks is not significantly impaired. The knockouts have a shorter lifespan than controls (LT₅₀ reduced from 26 to 20 months), which suggests that A₁R play an important protective role in mammalian tissues during aging [81,82]. In a follow-up study which included a six-arm radial tunnel maze test, A₁R^{-/-} mice were found to display normal spatial learning but to habituate more slowly to the test environment [83]. Thus, stimulation of A₁R may be required for habituation, i.e. suppression of the processing of irrelevant information.

3.3. Effects of Ethanol

Various effects of alcohol consumption appear to be mediated by adenosine and A₁R. Ethanol metabolism in the liver causes formation of acetate, which circulates in the body at millimolar concentrations and is finally incorporated into acetyl-coenzyme A with concomitant production of AMP and adenosine [84]. Acute administration of ethanol results also in inhibition of the facilitated diffusion of nucleosides [84,85]. The inhibitory effect is specific for one particular subtype of ENT (ENT1) [86,87] and can be observed both in cultured cells [84,85,88] and in cerebellar synaptosomes [89,90]. Thus, ethanol augments the rate of adenosine formation and reduces adenosine uptake. Both mechanisms may increase the extracellular levels of adenosine.

Initial attempts at measuring changes of adenosine levels within the brain after administration of ethanol or acetate were unsuccessful, probably because it is difficult to homogenize tissue, or to collect adenosine, while avoiding adenosine degradation [91,92]. Later analytical and microdialysis studies indicated that the levels of acetate and extracellular adenosine in cortical areas of rat brain are significantly increased (up to 4-fold) after administration of physiologically relevant doses of ethanol [93,94].

A functional relationship between ethanol and adenosine was suggested by the fact that dipyrindamole, an inhibitor of ENT1, promotes ethanol-induced motor impairment (EIMI) [95]. Increases of EIMI were also noted after administration of the ENT inhibitor dilazep or the AR agonist *R*-PIA, whereas the AR antagonist theophylline suppressed EIMI [96]. Several subsequent studies demonstrated that A₁R agonists accentuate and A₁R antagonists attenuate EIMI, when infused into the motor cortex [97] or striatum [98,99]. AR antagonists like theophylline or caffeine (Fig. 3), and lipophilic AR agonists like cyclohexyladenosine (CHA) modulate EIMI also after systemic administration. Experiments in which subtype-selective AR antagonists were administered either alone or in combination with agonists have indicated that the A₁R subtype is involved in modulation of EIMI, although a contribution of A₂R cannot be ruled out [97,99,100]. Co-administration of cAMP analogs, pertussis toxin or the (*R*)- and (*S*)-enantiomers of AR agonists with ethanol suggested involvement of G-protein-coupled A₁R and adenylate cyclase [101,102]. The importance of A₁R was proven by the fact that A₁R antisense, applied orally, systemically or directly into the striatum or cerebellum reduced the regional A₁R density and antagonized EIMI, whereas a mismatch control sequence had no effect [103,104]. A₁R in the motor cortex, striatum and cerebellum, but not in hippocampus [98] appear to affect EIMI via adenosine-induced decreases in glutamate release [105,106]

and/or changes of chloride conductance via chloride channels coupled to the GABA-benzodiazepine receptor complex [102,107].

Besides playing a role in EIMI, adenosine is involved in other physiological responses related to the consumption of ethanol. The ENT1 inhibitor dipyrindamole prolongs, and the AR antagonist theophylline reduces the duration of ethanol-induced sleep [95]. Ethanol and acetate also potentiate the anesthetic effect of sevoflurane and isoflurane through metabolically generated adenosine and stimulation of A₁R [108-111]. In humans, the AR antagonist caffeine reverses most of the sedating effects of ethanol (sleepiness, lack of alertness, impaired memory) but not ethanol-induced dizziness [112]. A recent study in rodents showed that ethanol promotes non-REM sleep but does not affect REM sleep. The somnogenic effect of ethanol is related to adenosine inhibition of wake-promoting neurons in the basal forebrain via A₁R. Bilateral microinjections of the selective A₁R antagonist DPCPX in this area reduce the effect of ethanol on non-REM sleep [113]. A study in which ethanol was administered to mice, either alone or in combination with selective AR agonists and antagonists, and animals were tested for anxiety in the elevated plus-maze, has indicated that activation of A₁R also mediates the anxiolytic effect of ethanol [114].

Adenosine and A₁R are not only involved in the motor impairing, sleep-promoting and sedating effects of ethanol, but also in counteracting the negative symptoms of ethanol withdrawal. Withdrawal signs in rats such as tremors and audiogenically induced seizures are suppressed by an A₁R agonist (CCPA), and this beneficial effect is blocked after co-administration of an A₁R antagonist (DPCPX) [115]. Other negative symptoms of ethanol withdrawal, such as hyperexcitability [116] and increased anxiety [117,118] are also ameliorated upon stimulation of A₁R. AR antagonists (DPCPX, caffeine) are neurotoxic when administered during ethanol withdrawal, particularly in female mice, and these neurotoxic effects are reversed by an A₁R agonist (CCPA). Sex differences observed for neurotoxicity of A₁R antagonists during ethanol withdrawal are probably related to N-methyl-D-aspartic acid (NMDA)-receptor-mediated downstream signaling which is more pronounced in females than in males [119,120].

Observed changes of A₁R densities in rodent brain during ethanol exposure appear to be dependent on the experimental paradigm, the radioligand which is used for binding assays and the brain area that is studied. Using the agonist [³H]R-PIA, increases of B_{max} without any change of K_d were noted in the cerebral cortex of rats, 15 min after administration of ethanol (1.5 g/kg, [96]). In a later study, the same authors noted that the observed increase (+40.7%) is transient, receptor densities returning to the control value within 60 minutes [121]. Chronic administration of ethanol is also accompanied by an increased binding (+23%) of the agonist 2-chloro-*N*6-cyclopentyladenosine ([³H]CCPA, Fig. 3) in rat cerebral cortex. This increase persists longer than the one observed after acute administration. Elevated receptor densities are observed 3, 12 and 24 h after the last consumption of ethanol, but the ethanol effect disappears after 3 to 6 days [122]. Increased binding of the A₁R agonist [³H]CHA was also noted in the cerebral cortex of mice following chronic administration of ethanol [123]. The increase is most pronounced after multiple episodes of ethanol withdrawal, and may be a compensatory inhibitory response to withdrawal seizures [124]. In contrast to the changes observed with A₁R agonists, cortical binding of the A₁R antagonist [³H]DPCPX is not affected by ethanol treatment [116,122]. Whereas most studies reported a transient increase of A₁R agonist binding upon acute or chronic administration of ethanol, one old [95] and one recent [125] study reported decreases of A₁R expression in some brain areas of rats. Decreased A₁R expression in the wake-promoting basal forebrain may be related to insomnia associated with ethanol withdrawal [125].

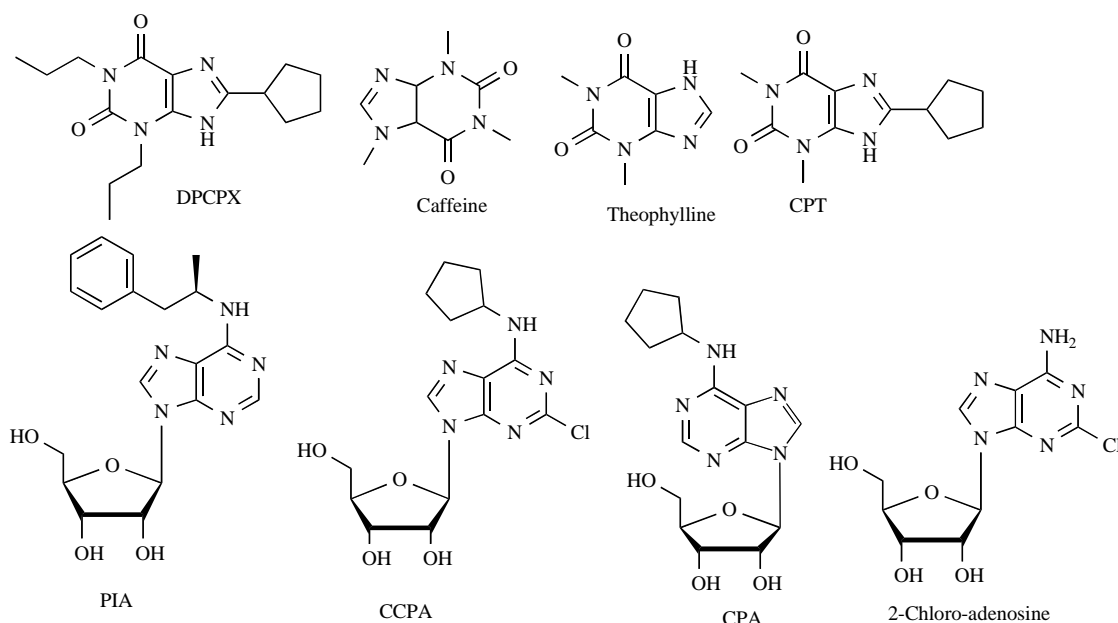


Fig. (3). AR ligands which are often used in binding studies. Top row: antagonists, bottom row: agonists.

Only a single PET study has examined changes of A₁R expression after acute exposure of animals to ethanol [126]. In that study, Wistar rats were treated with a combination of ethanol and the AK inhibitor ABT-702, and scanned with the A₁R ligand [¹¹C]MPDX. A striking (40-45%) increase of tracer distribution volume and binding potential was noted in target areas such as hippocampus, striatum and cerebral cortex, 20-90 min after treatment. Additional PET studies could be performed to study the effect of ethanol only, either in rodents or in the human brain.

3.4. Cerebral Ischemia

Levels of extracellular adenosine in the brain are dramatically increased during ischemia. In patients with transient ischemic attack (TIA) or stroke, a rapid rise of the plasma concentration of adenosine occurs in peripheral venous blood, presumably because of spillover from the brain, and this increase persists for days (TIA) or weeks (stroke) [127]. Extracellular adenosine in the brain inhibits synaptic neurotransmission including the release of excitatory amino acids [128,129], leading to a better matching of energy demand and energy supply in ischemic areas and a reduction of delayed excitotoxicity [130]. Through A₁R, adenosine promotes both the spontaneous electrical shutdown of the ischemic brain [131] and post-ischemic electrocortical burst suppression [132,133]. When levels of extracellular adenosine in mouse brain are reduced by transgenic overexpression of AK, postischemic cell death is increased [134].

The importance of A₁R stimulation was also evident in studies regarding ischemic preconditioning. When neurons or living animals are exposed to a short period of sublethal ischemia, they are better protected to a subsequent serious ischemic insult. This beneficial effect of preconditioning disappears in the presence of an A₁R antagonist like DPCPX, suggesting that A₁R stimulation is involved [135-139]. The mechanisms underlying induction of rapid tolerance by a sublethal ischemic insult are not fully understood. Increases in protein kinase C activity, mitogen-activated protein (MAP) kinase activity, Akt activity, nitric oxide production and mitochondrial K_{ATP} channels may all be involved [135-139]. Activation of presynaptic K⁺ channels *via* stimulation of A₁R may decrease evoked neurotransmitter release by hyperpolarization of the presynaptic membrane and thus improve resistance of the brain to ischemia.

In various animal and *in vitro* models, acute administration of A₁R agonists [140-143], inhibitors of adenosine uptake (propentofylline but not dipyridamole) [144], or inhibitors of AK [134] has been shown to result in protection against ischemic brain damage and reperfusion injury. Long-term treatment of gerbils with caffeine causes an upregulation of cerebral A₁R (10-17%) and greater resistance of neurons to ischemia induced by bilateral carotid artery occlusion [145]. Increasing the binding of adenosine to A₁R by administration of an allosteric enhancer also results in decreased brain damage after a hypoxic insult [146]. In contrast, acute administration of an A₁R antagonist such as DPCPX aggravates the consequences of cerebral ischemia [141,142,147].

Therapeutic application of A₁R agonists in stroke patients may be complicated by the fact that such compounds can have diametrically opposite effects after chronic and acute administration. Acute treatment with an A₁R agonist may reduce postischemic neuronal losses whereas chronic treatment with the same compound increases brain damage [142,148]. Other agonists are neuroprotective even during chronic treatment [149]. The underlying mechanisms are far from clear since the consequences of chronic administration of A₁R ligands can not always be related to up- or downregulation of A₁R [142].

Many studies have examined changes of A₁R expression in the brain of experimental animals after cerebral ischemia. In most studies, rapid decreases of A₁R mRNA and protein were observed during reperfusion [150-152] but in one study, decreases were noted at the mRNA but not the protein level [153]. These (slightly) discrepant findings may be due to the fact that different models of ischemia were employed and animals were examined at different intervals after the ischemic insult. Some changes appear only at intervals greater than 24 hours. Changes of A₁R expression during ischemia have been little examined, as opposed to changes during reperfusion. In a single study, increases of A₁R expression (at the mRNA and protein level) were noted during bilateral carotid artery ligation with a return to normal values immediately after reperfusion [154].

An interesting study from Japan examined changes of cerebral A₁R in cat brain following an ischemic insult, using PET and the radioligand [¹¹C]-MPDX. Decreases of A₁R were noted, and the magnitude of these losses increased with increasing severity of the

insult. The extent of ¹¹C-MPDX binding directly after reperfusion appeared to be a sensitive predictor of survival and disease symptoms during the follow-up period which lasted up to 2 months [155,156].

3.5. Epilepsy

Adenosine inhibits the release of excitatory neurotransmitters and suppresses cellular activity and energy demand. Based on this evidence, it has been proposed that adenosine is a powerful endogenous anticonvulsant substance [157]. In amygdala-kindled rats, intracerebrally administered A₁R agonists, such as 2-chloroadenosine (Fig. 3) and PIA, could indeed suppress seizures [158-162]. These substances were also effective in other animal models of epilepsy, such as entorhinal cortex kindling [163], piriform cortex kindling [164], hippocampal kindling [162,165], caudate nucleus kindling [162], intrahippocampal injection of kainic acid [166], intraperitoneal injection of pilocarpine [167] and hypoxia-induced convulsions [168]. The location of intracerebral administration is probably important for the therapeutic outcome [169]. In many studies, co-administration of A₁R antagonists was shown to result in a reduction of the anticonvulsant effect, which indicates that the A₁R subtype is involved in the beneficial action of the agonists.

Inhibitors of the enzyme AK can be systemically administered and can increase extracellular adenosine levels in metabolically active brain regions. Such compounds show similar anticonvulsant actions as A₁R agonists but lack the undesired peripheral side effects of these drugs [170]. AK is regionally overexpressed in epilepsy and this overexpression appears to contribute to the development and progression of seizure activity [134,171,172]. Local administration of adenosine by implanting encapsulated myoblasts could be a promising strategy for long term treatment of focal epilepsy [173].

Further evidence for the involvement of the A₁R in seizure suppression was obtained in animal models where A₁R antagonists were administered. In a cat model of epilepsy (general seizures induced by intracortical injection of penicillin), the A₁R antagonist CPT significantly prolonged the cycle period of seizures by increasing the duration of ictal discharge, in contrast to 7-aminobutyric acid or opioid peptides [174]. Adenosine may accumulate during the ictal phase and be cleared during the interictal phase in status epilepticus. In a rat model of epilepsy (electrically induced seizures), secondary seizures were prolonged after administration of an A₁R antagonist, and partial seizures were converted into generalized motor seizures [175]. In another rat model of epilepsy (i.p. administration of pilocarpine), the A₁R antagonists DPCPX and 3,7-dimethyl-1-propargylxanthine (DPMX) showed proconvulsant effects, by significantly reducing the latency to develop status epilepticus [167]. Theophylline also lowered the seizure threshold and prolonged hyperthermia-induced seizures in juvenile rats [176]. Using a selective A₁R antagonist (CPT), evidence was obtained that the inhibitory effect of low frequency stimulation of the perforant path on kindling acquisition in rats is mediated by stimulation of A₁R [177].

Changes of cerebral A₁R densities have been assessed both in animal models of epilepsy and in human brain post mortem. In a rat model of acute general seizure (i.p. injection of a convulsive dose of bicuculline), a widespread increase of A₁R (³H-CHA binding) was noted, which was particularly evident in hippocampus, amygdala, substantia nigra and septum [178]. In a mouse model of general seizure (pentylenetetrazol-induced convulsion) [179], A₁R were upregulated in most brain areas but downregulated in the basal ganglia [180]. In tissue samples acquired from patients with temporal lobe epilepsy, a 48% increase of A₁R density was observed in neocortex as compared to control samples from non-epileptic subjects [181].

However, in another study on human temporal lobe epilepsy, A₁R binding in temporal cortex was found to be reduced compared to normal postmortem controls [182]. A 15% decline of A₁R density was also noted in the basal ganglia and thalamic nuclei of genetic absence epilepsy rats from Strasbourg compared to wild-type littermates [183]. Even greater losses of A₁R were noted in the hippocampus and cerebral cortex of kainate-treated, hippocampus-kindled and amygdala-kindled rats, animal models of chronic epilepsy [184-187].

These conflicting results (either an upregulation or downregulation of A₁R) may be related to differences in the experimental setup in animal models and differences in patient selection in human studies. An acute seizure, or a limited number of seizures may be accompanied by upregulation of A₁R, or increased coupling of A₁R to G-proteins [188,189], as a protective mechanism enhancing the anticonvulsant effect of endogenous adenosine. However, chronic seizures may cause a significant death of neurons with accompanying losses of A₁R. The effects of partial and general seizures may also be different and the age (or developmental stage) of the subjects may affect the response of A₁R to kindling [178,190].

3.6. Traumatic Brain Injury

Studies in A₁R knockout mice have confirmed that this subtype plays an important role in suppressing neuronal hyperactivity. When normal and A₁R knockout mice were subjected to controlled cortical impact – an animal model of traumatic brain injury (TBI) – seizure scores were much higher in the knockouts and only the knockout animals developed lethal status epilepticus [191]. Similarly, unilateral hippocampal kainate injection caused non-convulsive status epilepticus in wild-type mice but severe convulsions and subsequent death of the animals in A₁R knockouts [192]. The authors concluded that activation of A₁R by adenosine is crucial in keeping an epileptic focus localized. Status epilepticus results in widespread adenosine release throughout the brain and suppression of the excitability of neurons remote from the epileptic focus *via* stimulation of A₁R. In A₁R knockout mice, this protective mechanism is lacking, so that severe convulsions and lethal status epilepticus can develop [192].

In another animal study on mild TBI, A₁R knockout mice were shown to display a 20-50% enhanced microglial response compared to their wild-type littermates. Moreover, stimulation of A₁R in BV-2 (immortalized mouse microglia) cells inhibited microglial proliferation [193]. Thus, A₁Rs appear to play an anti-inflammatory role in TBI-induced neuroinflammation.

To the best of our knowledge, no PET studies on changes of A₁R expression in epileptic patients or in animal models of epilepsy and TBI have been performed. Thus, there is considerable opportunity for imaging studies in this area.

3.7. Neuroinflammation

Besides its well-known function as an inhibitor of neuronal overactivity and its involvement in the regulation of sleep, adenosine has been proposed to act as an endogenous anti-inflammatory agent [194]. In the CNS, A₁Rs are expressed not only on neurons but also on glia [195]. A₁R stimulation in microglia, the endogenous immune cells of the brain, may have both pro- and anti-inflammatory effects, depending on the presence or absence of other factors in the environment, such as phorbol 12-myristate 13-acetate [196]. An early study reported that simultaneous activation of both A₁R and A_{2A}R stimulates microglial proliferation, whereas selective A₁R or A_{2A}R agonists when applied alone have no effect [195]. However, later studies have indicated that in most situations, A₁R agonists suppress neuroinflammation.

In cultured astrocytes, CCPA enhanced the release of nerve growth factor [197], a compound which is essential for neuronal protection and a suppressor of neuroinflammation within the specific environment of the brain [198]. In plasmacytoid dendritic cells, adenosine has been suggested to play a dual role: first, chemotaxis to the site of inflammation is stimulated *via* A₁R, then, the extent of the inflammatory response is limited by inhibition of the production of several cytokines such as interleukin-6, interleukin-12 and interferon- α [199].

The importance of A₁R in controlling neuroinflammation was highlighted by studies of experimental allergic encephalomyelitis (EAE). Mice lacking A₁R developed a much more severe, progressive-relapsing form of EAE than their wild-type littermates. Reduced densities of A₁R in microglia of wild-type mice were observed during the neuro-inflammatory phase of EAE. Upregulation of A₁R by treatment of normal mice with caffeine reduces EAE severity, and this beneficial effect of caffeine can be enhanced by concomitant administration of an A₁R agonist [200]. In a rat model of neuroinflammation (EAE induced by guinea pig spinal cord homogenates), chronic caffeine treatment results in up-regulation of A₁R and attenuation of EAE pathology [201]. Experimentally induced neuroinflammation by chronic infusion of lipopolysaccharide (LPS) into the fourth ventricle of young rats, and natural microglia activation in aged rats are also attenuated after treatment of animals with caffeine [202]. In contrast to EAE (an animal model of chronic neuro-inflammation which is associated with a downregulation of A₁R), acute inflammation of mouse brain after administration of LPS leads to increased A₁R expression in cortical areas and this response is dependent on the transcription factor NF-kappaB [203].

An interesting study examined the role of A₁R in brain tumors. Gliomas do not consist only of tumor cells but to a large extent (up to 30%) also of microglia and macrophages. Microglial cells accumulate particularly at the tumor rim. In the tumor environment, microglia acquires a different phenotype which is associated with expression of matrix metalloprotease II (MMP2). After acquisition of this novel phenotype, the microglial cells do not suppress but can actually promote tumor growth and invasion since these processes are MMP2-dependent. Experimental glioblastomas grew more vigorously and were associated with larger numbers of microglial cells in A₁R knockout mice than in wild-type mice. In wild-type animals, A₁R were up-regulated in microglia in contact with tumor cells but not in the rest of the brain. When glioma and microglia cells were cultured together *in vitro*, A₁R agonists suppressed the growth of tumor cells, but in the absence of microglia, the same compounds stimulated tumor growth. Thus, adenosine attenuates glioblastoma growth, acting through microglial A₁R [204].

Changes in cerebral A₁R density caused by glioma invasion have been examined with the tracer [¹⁸F]CPFPX and PET, both in an animal model (F98 glioma-bearing rats) and in a patient with recurrent glioblastoma multiforme. In the animal model, A₁R were also quantified by *in vitro* and *in vivo* autoradiography and immunohistochemical analysis. A₁R were shown to be upregulated (by 36 to 46%) in a zone directly surrounding the tumor, and to be localized in activated astrocytes [205]. It would be interesting to study changes of A₁R expression with microPET in animal models of neuroinflammation (e.g., the EAE model, the LPS model, or virally induced encephalitis). Such studies have not yet been reported.

3.8. Human Diseases of the CNS

3.8.1. Alzheimer's Disease (AD)

Since A₁R signaling can be neuroprotective, many investigators examined changes of regional A₁R density in human brain in neurodegenerative disorders. Initial autoradiographic studies were focused on AD and the hippocampus, a brain region involved in

memory. A significant loss of A₁R was observed in the CA1 (-47%) and dentate gyrus molecular layers (-46%) of the hippocampus but not in the CA3 [206]. Another study reported 40-60% decreases in all areas of the hippocampus, the most striking losses occurring in the molecular layer of the dentate gyrus [207]. Still another investigation reported substantial decreases in the dentate gyrus but A₁R densities close to normal in CA1 and CA3 [208]. The former two investigations used the A₁R agonist [³H]CHA whereas the last study employed the antagonist ligand [³H]CPDPX.

Binding of an A₁R agonist ([³H]R-PIA) and antagonist ([³H]CPDPX) in the AD hippocampus were later directly compared. Both ligands detected prominent losses of A₁R in the dentate gyrus. Decreased agonist binding was observed in CA1 and outer layers of the parahippocampal gyrus, whereas losses of antagonist binding were noted in the subiculum and CA3. These decreases reflected reductions of receptor number (B_{max}) rather than reductions of the affinity (K_D) of A₁R for the radioligands [209].

In the striatum of AD patients, 30-36% losses of A₁R were noted both in the caudate nucleus and putamen, and these losses appeared to parallel the decrease of choline acetyl transferase [210].

An immunohistochemical study from Spain showed that A₁R are redistributed in the hippocampus and cerebral cortex of AD patients in comparison to age-matched controls. A₁R accumulate in degenerating neurons and are co-localized with β -amyloid in senile plaques and with tau in neurons with tau deposition. Studies in the human neuroblastoma cell line SH-SY5Y indicated that A₁R stimulation increases the production of soluble forms of amyloid precursor protein, and the phosphorylation and translocation of tau to the cytoskeleton. By inhibiting the deposition of amyloid and enhancing the translocation of tau, A₁R stimulation may slow down the process of neurodegeneration [211].

An upregulation of A₁R was detected in the frontal cortex of AD patients (both at early and advanced stages of the disease), using quantitative autoradiography and the radioligand [³H]DPCPX [212]. However, a PET study in AD patients and elderly normal subjects indicated significant decreases of the binding potential of [¹¹C]MPDX in the temporal and medial temporal cortices and thalamus of the patients with no significant change in other areas of the brain [213] (Fig. 4). Discrepant findings in human studies may be related to the facts that different patient groups were selected (early vs. late onset AD), different brain regions were examined, and different techniques were used for quantification (immunohistochemistry, ligand binding). Moreover, a transient upregulation of A₁R may be followed by an eventual receptor loss.

3.8.2. Pick's and Creutzfeldt-Jakob Disease

In the frontal cortex of individuals with Pick's [214] and Creutzfeldt-Jakob [215] disease, similar increases of A₁R density were noted as in patients with AD, using quantitative autoradiography and [³H]DPCPX. No PET studies with [¹¹C]MPDX or [¹⁸F]CPFPX in such patients have been reported.

3.8.3. Multiple Sclerosis (MS)

Several studies have reported changes of AR signaling in patients with MS. Plasma levels of TNF- α in such patients are significantly higher and levels of adenosine are significantly lower than in control subjects. Stimulation of peripheral blood mononuclear cells (PBMC) with a selective A₁R agonist (R-PIA) inhibits the mitogen-stimulated production of TNF- α in healthy subjects but not in MS patients, whereas the opposite is observed for the mitogen-stimulated production of interleukin-6 [216]. A₁R densities in PBMC and in brain tissue from MS-patients are significantly decreased (by 53% and 49%, respectively) compared to age-matched controls [217]. Thus, both A₁R densities and the coupling of A₁R to cytokine signaling systems appear to be altered in MS. However, no PET studies with A₁R ligands have been performed in this patient group.

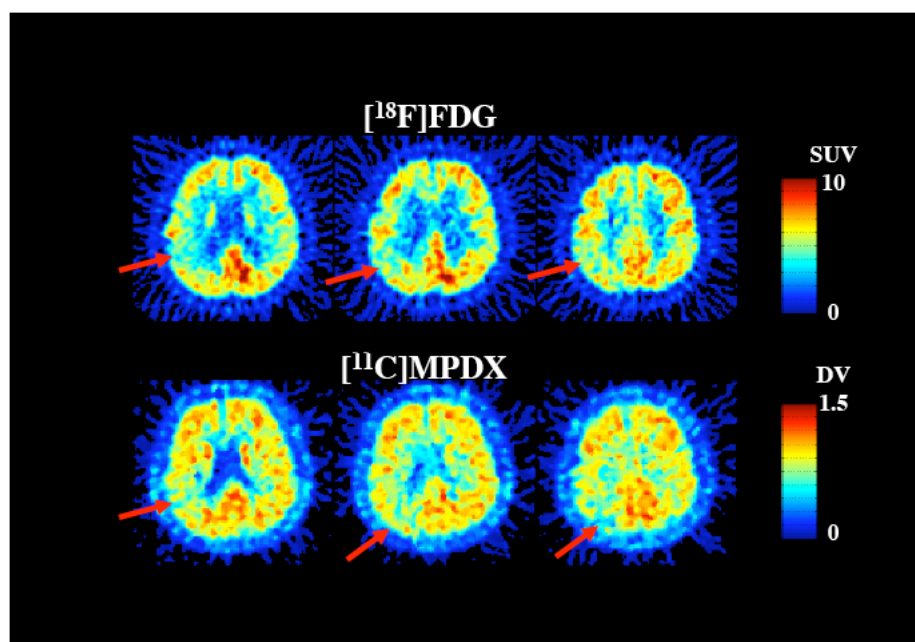


Fig. (4). PET scans of the brain of an Alzheimer disease patient, made with [^{18}F]FDG (top row) and [^{11}C]MPDX (bottom row). Distribution patterns of [^{18}F]FDG and [^{11}C]MPDX are similar, but a statistical parametric analysis revealed that [^{11}C]MPDX PET was not coupled with hypometabolism in the posterior cingulate gyrus.

3.8.4. Parkinson's Disease

In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, the drug paeoniflorin proved capable of reducing neurodegeneration and inhibiting neuroinflammation by activation of A₁R [218]. Thus, modulation of neuroinflammation *via* A₁R may be a novel approach towards treatment of neurodegenerative disease.

Deep brain stimulation (DBS) is frequently applied for the treatment of movement disorders and may also benefit individuals with psychiatric disease. Recently, it was shown that the mechanism underlying the beneficial effect of DBS is a local release of ATP which is extracellularly metabolized to adenosine and suppresses tremor *via* A₁R. The effect of DBS can be mimicked by intrathalamic infusion of A₁R agonists. On the other hand, A₁R antagonists like caffeine can trigger or exacerbate essential tremor [219].

3.8.5. Schizophrenia

A partial loss of A₁R during birth and a corresponding reduction of the control of dopamine activity in later life has been proposed to play a role in the inhibitory deficit in schizophrenia [220,221]. Association between a single nucleotide polymorphism of the A₁R gene (rs3766553) and schizophrenia was indeed noted in a Japanese population [222]. PET studies of cerebral A₁R in patients with schizophrenia have not yet been performed.

4. PHARMACODYNAMIC STUDIES

4.1. A₁R (ant)agonists

PET has important applications in the study of pharmacodynamics, i.e. assessment of the effects of drugs in the healthy and diseased body, including their mechanisms of action and the relationship between drug concentration and effect. During the development of CNS drugs, PET is often used to measure the dose-dependent occupancy of target receptors in the human brain by a non-radioactive test compound. PET is also capable of measuring the metabolic response of tissues to treatment

quantitatively, repeatedly and noninvasively, both in experimental animals and humans.

Only a few PET studies regarding A₁R occupancy by cold antagonists have been reported in the literature. An interesting paper from Germany showed that the binding of ^{18}F -CPFPX in rat brain is reduced after treatment of animals with caffeine, shortly before injection of the tracer. At a dose of 4 mg/kg, corresponding to consumption of three to four cups of coffee by a human being, tracer binding was strongly (ca. 50%) suppressed, suggesting ~70% receptor occupancy [223]. Another study showed that ^{18}F -CPFPX bound to A₁R in human brain can be dose-dependently displaced by injecting non-radioactive CPFPX [224]. A microPET study from our own group has indicated that A₁R in rat brain are almost completely occupied after administration of DPCPX (3 mg/kg, i.p.), 20 min before injection of ^{11}C -MPDX [126].

Thus, the dose-dependent occupancy of A₁R in the brain by test drugs may be assessed by PET. Dose-dependent effects of A₁R agonists (or AK inhibitors, see 4.2) on organ metabolism could be assessed as well, using the radiolabeled glucose analog [^{18}F]FDG, but such studies have not yet been reported.

4.2. AK Inhibitors

AK regulates intra- and extracellular adenosine concentrations by phosphorylation of adenosine to AMP. Although ADA also removes adenosine by converting it to inosine, the reaction catalyzed by AK is the most important pathway of adenosine removal under physiological conditions [225]. Since the major adenosine-specific nucleoside carrier acts as a non-concentrative, bi-directional, facilitated diffusion transporter, adenosine transport is driven by the concentration gradient across the cell membrane. Inhibition of AK raises intracellular adenosine, diminishes the concentration gradient and decreases the cellular uptake of adenosine, resulting in increased extracellular adenosine concentrations, particular under pathophysiological conditions and in tissues or brain regions where the formation of adenosine is increased by net catabolism of ATP [226-228]. An *in vivo* study in rats has shown that AK inhibitors (AKIs) augment the increases of

extracellular adenosine during excitotoxic insults in the striatum but do not affect striatal adenosine levels in vehicle-treated controls [228]. Thus, AKIs represent a strategy for potentiating the protective actions of endogenous adenosine during tissue trauma [229]. This therapeutic approach may be advantageous in cases where not a single subtype (e.g., A₁R) but rather a multiplicity of AR subtypes should be stimulated for the beneficial effect [230].

Three applications of AKIs have been proposed. First, intrathecally administered AR agonists [231-233] and AKIs [232-234] have antinociceptive effects in experimental animals. These effects are counteracted by selective A₁R antagonists but not by A_{2A}R antagonists suggesting involvement of A₁R stimulation [228,232,235,236]. Novel AKIs such as ABT-702 and A-286501 can also be given subcutaneously or orally and are then effective in animal models of acute, inflammatory and neuropathic pain [235,237-239]. Substantial interest in the use of AKIs was raised by the fact that these compounds can be equally effective as morphine for the suppression of pain but show less potential to develop tolerance [238,240,241]. The use of AKIs rather than AR agonists allows an adequate separation between antinociceptive and motor-impairing effects [242].

Second, AR agonists and AKIs have shown anti-inflammatory efficacy in various animal models of acute and chronic inflammation [238,239,243-248].

A third potential application of AKIs is in the treatment of epilepsy. These compounds suppress seizures in rodent models, such as the bicuculline-induced seizure [249] and the maximal electroshock [170,250,251] model, presumably *via* an interaction of adenosine with cerebral A₁R. For that reason and because upregulation of the enzyme AK is involved in epileptogenesis [171], AKIs are considered promising anticonvulsants [170,252,253].

If a ligand could be developed of which the binding is sensitive to competition by endogenous adenosine, PET imaging could be applied to assess changes of extracellular adenosine induced by AKIs.

CONCLUSION

The purine nucleoside adenosine and its A₁R are implied in many physiological functions, such as regulation of the sleep-wake cycle, aggression, and anxiety, habituation of animals to a novel environment, protection of cells against the negative consequences of hypoxia and ischemia. Adenosinergic signaling is also implied in ethanol-induced motor impairment, and withdrawal symptoms after ethanol abuse. Stimulation of A₁R has anticonvulsant and anti-inflammatory actions. Changes of A₁R expression have been noted in animal models of epilepsy, the micro-environment of brain tumors, and various diseases of the human CNS such as AD, Pick's disease, Creutzfeldt-Jakob disease, MS and schizophrenia. Since appropriate ligands for PET imaging of A₁R are now available, PET can be applied to elucidate the role of A₁R in the normal and diseased human brain and to study the pharmacodynamics of A₁R agonists and AKI.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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